



Mosquito repellent propensity of hexane extract of *Cymbopogon citratus* Stapf. (Poaceae), lemongrass, cream and emulgel formulations

Omoshalewa M. ADESOLA¹, Olubunmi J. OLAYEMI², John ALFA^{1*}

¹Department of Pharmaceutics & Pharmaceutical Technology, Bingham University, Karu, Nasarawa State, Nigeria.

²Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

Received 5th May 2024; Accepted 29th July 2024

Abstract

Malaria is a global threat affecting majorly the African continent with Nigeria having the highest burden of deaths from the disease. Prophylactic measures in addition to curative treatments are strategies used for eradication of malaria. This study aims to develop topical mosquito repellent from lemongrass (*Cymbopogon citratus*). Lemongrass extract was obtained by Soxhlet extraction using *n*-hexane, it was formulated as lemongrass extract cream (LGCM) and emulgel (LGEM) at 1 and 2% w/w concentrations. Organoleptic properties, spreadability, pH and skin irritation test were evaluated. Percentage mosquito repellency (R) and complete protection time (CPT) assay using the animal (rodent) model were determined and compared with N, N-diethyl-meta-toluamide (DEET). Results showed all the formulations were non-gritty, homogenous, relatively stable upon storage for 30 days at room temperature except for LGCM 1. They were non-irritant to the skin; pH was in range of 5.3 – 8. Spreadability was optimum with LGCM, LGEM 1 and 2% w/w. Mosquito repellency was concentration dependent and > 80% in LGCM 2 and LGEM 2 but emulgels exhibited better repellency. CPT was 4, 5 and 8h for LGCM 2, LGEM 2 and DEET respectively. This study shows that LGEM 2% may be considered as an efficient topical mosquito-repelling formulation.

Keywords: *Cymbopogon citratus*; Lemongrass; Cream; Emulgel; Mosquito repellence

INTRODUCTION

Malaria is a major global public health issue affecting majorly the African continent resulting in 94% of global malaria cases and about 95% deaths. Over half of the global burden of malaria deaths worldwide is accounted for by Nigeria (26.8%), Democratic Republic of Congo (12.3%), Uganda (5.1%) and Mozambique having about 4% [1]. Protozoan parasites of the genus *Plasmodium* particularly *Plasmodium falciparum* is

responsible for the malaria disease, the parasites are transmitted through the bite of the infected female Anopheles mosquito. There is no permanent cure to malaria, as such, prophylactic measures against malaria are being exploited to prevent its infestation, one of such means is by deterring contact of the mosquito on the skin surface [2]. Thus, protection by use of mosquito repellents on exposed skin surfaces is one of the effective approaches to diminish the incidence of

*Correspondence. E-mail: john.alfa@binghamuni.edu.ng Tel: +234-8036548373.

ISSN 0189-8442

2024. Published by Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. Under Creative Commons Attribution-Non-Commercial 4.0 International License. <https://creativecommons.org/licenses/by-nc/4.0/>

malaria. Mosquito repellents are topical substances applied to the skin or in some cases, on clothes, which prevents mosquitoes from landing on the skin or surfaces. They work by masking the human scent or by exuding a scent that mosquitoes would avoid [3].

Currently, most of the available mosquito repellents are synthetic which produce some toxic effects like nausea, vomiting, hypotension, encephalopathy, seizure, coma, and ataxia from exposure on skin [4]. There are reports of natural remedies, including plant parts, been effective in retarding mosquito bites [5]. Plant-based remedies are an integral part of the healthcare system as alternative treatment and are being used by about 80 % of the populace in developing climes [6] due to ease of accessibility, affordability and purported natural safety [7]. As with treatment or management of diseases, plant-based repellents have recently gained interests because they are a rich source of many bioactive chemicals that are effective as insecticidal and can be used as mosquito repellents [8]. Traditionally, they are still been used in rural communities of tropical regions as a means of protection from mosquito bites. Specific plant parts are simply hung around houses and have proven to be effective in repelling insects including mosquitoes [9].

Cymbopogon citratus Stapf., is one of the most familiar species of the genus *Cymbopogon* from the family Poaceae (Gramineae) also known as the “sweet grass family”. It is a plant native to Asia (Indochina, Indonesia, and Malaysia), Africa (Nigeria, Ghana, Kenya, South Africa, Democratic Republic of Congo, Angola, Madagascar and Uganda), North America and South America, but is now widely cultivated in temperate and tropical regions of the world [10]. It is called “Lemongrass” in English, “Citronelle” in French and “Citronella” in Portuguese. The plant is widely cultivated in the Southern parts of Nigeria and is known by several names; it is

called “Eti” by the Edo tribe, “Ikon eti” by the Efiks, “Tsauri” by the Hausas, “Achara ehi” by the Igbos, “Kooko oba” by the Yorubas [11] and “Egbe ihiolo” (nasal congestion grass) by the Igalas. Lemongrass is used widely for its culinary benefits; it is commonly consumed as teas especially in African and Latin American countries, they are also made into soups and curries [12]. The leaves possess a lemon-fragrant essential oil which has been shown to be responsible for many of its activities. In addition, the leaves contain proteins, vitamins and minerals in abundance [13].

Therapeutic benefits of the plant include its use as antibacterial, antifungal, anti-amoebic, anti-filarial, anti-inflammatory, antimalarial, neuro-behavioral effects, anti-rheumatic among others [14-18]. However, there are anecdotal reports of the use of lemongrass as repellent in preventing mosquito or insect bites generally.

The repellent activity of lemongrass plant is basically ascribed to the active components of its essential oil such as geranial, neral, geraniol, citronellol, citronellal and elemol which are present in various compositions [19].

Literature search reveals studies that have exploited lemongrass in different forms as mosquito repellent. An earlier study [20] developed ointment and cream formulations of lemongrass oil and reported that the formulations had considerable activity against mosquito bites as that of a commercial mosquito repellent formulation. Another study, exploited development of fragrance from lemongrass oil found the aromatic and deodorizing properties of the fragrance to be comparable to that of a standard fragrance [21]. In a different study [22], mosquito repellent cream from essential oils of clove, lemongrass and citronella was developed and found to be effective in repelling mosquitoes. A more recent study formulated gels, incense sticks and liquid for plug-in device from combination of essential oils from lemongrass,

cedarwood, lavender, and rosemary and found the different formulations are effective as protection against mosquito bites [23]. Another study [24] developed an insecticide from extracts of the stem and leaves of lemongrass and found that extracts from the leaves showed significant increase in mortality rate of *Aedes aegypti*. A related study [25] evaluated the making of mosquito repellents from wastes obtained from processing citronella oil. They reported the possibility of developing mosquito coils from these solid wastes with ability to repel mosquitoes.

Many repellent formulations have been prepared ranging from creams to gels, lotions and sprays. Creams are semisolid formulations prepared as oil in water (o/w) or water in oil w/o emulsions. They are easy to apply to the skin, have a soft feel on the skin, have good permeability and are aesthetically appealing [26]. The novel preparation in this project is the preparation of an emulgel-based mosquito repellent. Emulgels are semisolid preparations like creams but they consist of the mixture of an emulsion and a gel; they are particularly effective in delivery of hydrophobic drugs than other common topical systems. These formulations are good for preparation of repellents because they can be easily spread over the skin, are non-greasy, are cosmetically appealing, possess emollient properties and do not leave residues after application [27].

Despite the fact that most studies report the activity of lemongrass oil as an insect repellent, nonetheless, this study is set to investigate the mosquito repellent activity of the crude extract of the whole leaves. The aim is to develop cream and emulgel formulations containing the *n*-hexane extract of *Cymbopogon citratus*, evaluate their physicochemical properties and compare the mosquito repellent activities of the formulations.

EXPERIMENTAL METHODS

Materials. *Cymbopogon citratus* leaves obtained from a garden in Karu, Nasarawa state, Nigeria. Hexane (Loba Chemicals Ltd, India), Liquid paraffin (Analar grade), Triethanolamine (Meru Chem PVT, Ltd, India), Methyl cellulose (BDH Chemicals Ltd, UK), Emulsifying wax (Fisher Chemicals, USA), Sodium benzoate (BDH Chemicals Ltd, UK), DEET cream (Dabur Pharma Ltd, India). Dried *n*-hexane extract of *Cymbopogon citratus* prepared in the laboratory of Bingham University Karu, Nasarawa State.

Animals. Male Wistar rats weighing between 150 and 200 g were used for the experiment. The rats were obtained from the Animal Facility Centre of the Department of Pharmacology, Bingham University Karu, Nasarawa State, Nigeria. They were subsequently kept in cages under ambient conditions (natural lighting conditions) and were given access to food and drinking water *ad libitum*. The protocol of the study was approved by the Animal Care and Ethics Committee of the Department of Pharmacology Bingham University Karu, Nasarawa state.

Collection and identification of plant material. Fresh leaves of *Cymbopogon citratus* were obtained from a garden in Karu, Nasarawa state, Nigeria. The leaves were authenticated and identified by a Taxonomist and given a voucher number (DPHBHU 0051). The leaves were cleaned, dried at room temperature for 14 days then size-reduced and powdered.

Preparation of *Cymbopogon citratus* (Lemongrass) leaf extract. The powdered dried Lemongrass leaves (300 g) were placed in the thimble chamber of the Soxhlet apparatus. Extraction was carried out using *n*-hexane (1.5 L) at a ratio of 1:5 (leaves:solvent) for 72 hours at 65°C. The extract was concentrated by evaporation at 65°C for about four hours and then air-dried for 48 hours. The

resulting extract was pulverized, stored in an airtight container and placed in a desiccator at room temperature until further use.

Preparation of cream formulations containing Lemongrass extract. The composition of ingredients used for preparation of cream formulations containing the prepared Lemongrass extract is presented as Table 1. An earlier documented method [28] was modified and adopted; the aqueous phase was prepared by mixing quantities of triethanolamine, sodium benzoate and water at 70°C. The oily phase was prepared by melting appropriate amounts of liquid paraffin and emulsifying wax together over a water bath at 70°C then the lemongrass extract was mixed into the oily phase by stirring. The oily phase was incorporated into the aqueous phase with continuous stirring until the formulation was completely homogenized and cooled. The creams were packaged in air-tight containers and stored at room temperature for further analysis. Other batches were prepared in a similar manner using the appropriate ingredients in Table 1.

Preparation of emulgel formulations containing Lemongrass extract. The composition of ingredients used for preparation of emulgel containing the prepared Lemongrass extract is presented as Table 1. Here, a modified method [29] was adopted. The gel base was prepared by dissolving appropriate quantity of methylcellulose in water by stirring with a magnetic stirrer; sufficient quantity of triethanolamine was added until the gel was formed. The oil phase of the emulsion was prepared by melting emulsifying wax over a water bath heating system up to 70°C and mixing with liquid paraffin at same temperature; the appropriate amount of lemongrass extract was incorporated into the wax-liquid paraffin mix by stirring with the magnetic stirrer. The preservative; sodium benzoate was dissolved in distilled water at 70°C to make the aqueous phase. The oil and aqueous phases were

maintained at 70°C; the oil phase was gradually dispersed into the aqueous phase by continuous stirring until a stable emulsion was formed. This emulsion was combined with the gel in the ratio 1:1 and stirred until the emulgel was formed. The emulgel was packaged in suitable containers and kept at room temperature until required. Other batches were prepared in a similar manner using the appropriate ingredients in Table 1.

Evaluation of prepared cream and emulgel formulations of lemongrass extract

Organoleptic evaluation. The different formulations were physically analysed by visual inspection for colour, odour, homogeneity, grittiness, washability, skin feel, phase separation and cosmetic acceptability.

Determination of pH. The pH of the formulations was determined using a digital pH meter (PHS-25, England), three (3) determinations were made and the average was recorded.

Determination of spreadability. A modified method was adopted [28]. Weighed quantity of the formulation; 25 mg (M) was sandwiched between two slides, a standard weight (10 g) was placed on the covered slides for one (1) minute to allow the formulation to spread maximally. The extent to which the formulation spread was measured (cm) without removing the upper slide, the time taken to separate the upper slide from the lower slide (T) was recorded. Spreadability was computed using the expression:

$$MxL/T \dots \dots \dots (1)$$

Determination of microbial load. Analysis of microbial load of the formulations was determined using nutrient agar. One (1) gram of the formulations was streaked on the agar plate. The plates were inverted and incubated at 37°C for 24 hours; afterwards the plates were placed on a colony counter where the number of colony-forming units was determined.

Skin irritation test. Male rats were used for this test. The hairs on the back of each of the rats were shaved 24 hours prior to the test, 200 mg of the preparations were applied to the shaven skin of the rats (n=3). Another group of rats (n=3) on which the preparations were not applied were placed in different cages to serve as negative control. After the exposure period, skin sensitivity observed as changes in skin colour, erythema or oedema were noted and recorded using the Draize scoring criteria [30].

Mosquito repellent assay. Mosquito repellent activity was assessed using unbred mosquitoes and Wistar rats housed in a home-made cage. Modification of the method of Banik *et al.*, [31] was adopted to obtain the mosquitoes used in this study; unbred mosquitoes were harvested from a swampy location using a tiny net while flying over the swamp. An earlier method [32] as adopted from the WHO protocol [33] was used to investigate the repellency activity of the prepared formulations. Twenty-eight (28) male Wistar rats were divided into seven (7) groups with 4 animals in each group as follows; Group I: negative control group which received no treatment (NC), Group II: treated with formulation LGCM 1, Group III: treated with formulation LGCM 2, Group IV: treated with formulation LGEM 1, Group V: treated with formulation LGEM 2, Group VI: treated with formulation E3 and Group VII: treated with commercial product; DEET (N, N-Dimethyl-meta-toluamide).

The rats were picked out of their cage then the formulations were applied on shaven ventral skin of the rats before the animals were exposed to mosquitoes in a home-made box/cage (with an opening on one side, the opening was fitted with net to allow for ventilation). The host-seeking behavior of the mosquitoes was determined by placing animals in the negative control group in the box and counting the number of mosquitoes that bite the shaven skin in not less than 3 min [34]. The number of mosquito bites observed

on the shaved skin of treated rats (T) and those observed in the control animals (C) were used to determine the repellent effect of the formulations. Repellency (R) was calculated using the equation:

$$R \% = \frac{C - T}{C} \times 100 \dots \dots \dots (2)$$

Complete protection time (CPT). Complete protection time was measured as the time taken between application of the repellent formulations and first appearance of bite marks on the treated rats.

RESULTS AND DISCUSSION

Physical and chemical properties of the cream and emulgel formulations of extract of lemongrass. Four (4) formulations were prepared using lemongrass extract; 2 of them are cream formulations and the other 2 are emulgel formulations. Physical properties of these formulations are displayed in Table 2. The formulations containing 1% of the extract; either cream LGCM or emulgel LGEM, were observed to be light green in color while formulations LGCM 2 and LGEM 2 were a darker shade of green. This increase in color intensity is the effect of increased extract concentration in these formulations. All the formulations had characteristic pleasant smell due to the extract; they were soft, opaque, non-gritty and homogenous in appearance. The smooth and homogenous texture indicates there was uniform distribution of all the ingredients into each other signifying that these preparations can be easily and evenly applied on the skin without.

Application of LGEM 1 and LGEM 2 on the skin gave a cooling sensation characteristic of emulgels. All the preparations were cosmetically appealing and got easily washed off the skin under running water. There was no apparent sign of physical phase separation in all the LGCMS and LGEMS 24 hours post preparation. However, phase separation was observed upon storage for 30 days at room temperature in only formulation

LGCM 1; the cream was observed to have cracked and lost its aesthetic appeal. Generally, after the study storage period, the emulgels had better physical appearance than the creams. This suggests that emulgels of Lemongrass extract presents more stable and aesthetically acceptable product than the

corresponding cream formulations. This can be attributed to the presence of three-dimensional gel structure in emulgels which decreases surface interfacial tension of the formulations thereby increasing viscosity which stabilizes the formulation.

Table 1: Composition of ingredients for preparation of Lemongrass cream (LGCM) and emulgel (LGEM) formulations

Ingredient/Batch	LGCM 1	LGCM 2	LGEM 1	LGEM 2
Lemongrass extract (g)	1.00	2.00	1.00	2.00
Methylcellulose (g)	-	-	3.00	3.00
Emulsifying wax (g)	7.25	7.25	7.25	7.25
Liquid paraffin (mL)	25.00	25.00	25.00	25.00
Sodium benzoate (g)	1.00	1.00	1.00	1.00
Triethanolamine (mL)	1.50	-	1.50	1.50
Water to (mL)	100	100	100	100

LGCM 1 = Lemongrass cream containing 1 % w/v extract, LGCM 2 = Lemongrass cream containing 2 % w/v extract, LGEM 1 = Lemongrass emulgel containing 1 % w/v extract, LGEM 2 = Lemongrass emulgel containing 1 % w/v extract

Table 2. Physical properties of cream and emulgel formulations of lemongrass extract

Batch	LGCM 1	LGCM 2	LGEM 1	LGEM 2
Color	Light green	Dark green	Light green	Forest green
Odor	Pleasant	Pleasant	Pleasant	Pleasant
Homogeneity	+++	+++	+++	+++
Nature	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Grittiness	None	None	None	None
Skin feel	Good	Good	Cool	Cool
Washability	+++	+++	+++	+++
Phase separation (24 h)	-	-	-	-
Phase separation (30 days)	+	-	-	-

+++ = very homogenous, easily washable, + = phase separation, - = no phase separation

Table 3: Physicochemical properties of cream and emulgel formulations of lemongrass extract

Batch	pH	Spreadability (g.cm/sec)
LGCM 1	7.77 ± 0.02	23.5 ± 0.30
LGCM 2	5.39 ± 0.03	23.5 ± 0.60
LGEM 1	7.90 ± 0.02	17.5 ± 0.40
LGEM 2	7.84 ± 0.01	17.5 ± 0.30

Table 4: Microbial load in lemongrass creams and emulgels

Batch	TAMC (cfu/g)	<i>S. aureus</i>	<i>P. aeruginosa</i>
LGCM 1	< 10 ²	absent	Absent
LGCM 2	< 10 ²	absent	Absent
LGEM 1	< 10 ²	absent	Absent
LGEM 2	< 10 ²	absent	Absent

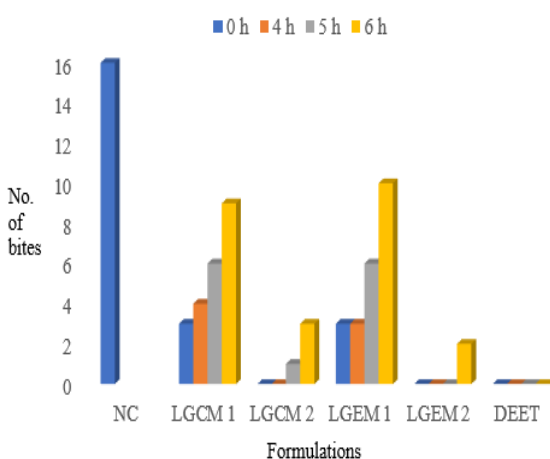
TAMC = total aerobic microbial count

Table 5: Skin irritation parameters on animal skin

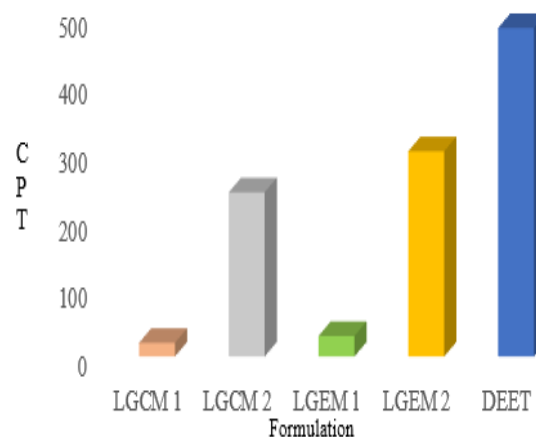
Batch/Reaction	Erythema	Oedema
LGCM 1	0/3	0/3
LGCM 2	0/3	0/3
LGEM 1	0/3	0/3
LGEM 2	0/3	0/3

Table 6: Mosquito repellency assay of lemongrass creams and emulgels

Batch	repellency (%)			
	0 (h)	4 (h)	5 (h)	6 (h)
LGCM 1	81.3	75	62.5	43.8
LGCM 2	100	100	93.8	81.3
LGEM 1	81.3	81.3	62.5	37.5
LGEM 2	100	100	100	87.5
DEET	100	100	100	100

**Figure 1:** Number of mosquito bites observed after application of different lemongrass extract formulations, the standard (DEET) and the negative control (NC)

The skin's protective acid mantle is slightly acidic which acts as a barrier against microorganisms that may penetrate the skin. It is therefore, necessary to use topical preparations whose pH are as near as possible to the physiological pH of the skin to prevent diverse skin problems [35]. Typical pH of the skin is about 5.5 and 6.5 where normal moisture and microbial habitats are maintained but the stratum corneum has been reported to tolerate wider pH ranging between 3 and 9 [36]. However, preparations with higher pH (> 9) are dangerous to the skin because they cause

**Figure 2:** Complete protection time of the various formulations against mosquito bites

dryness and irritation [37]. Table 3 shows the pH of all the preparations ranged between 5.39 and 7.90 which is within the acceptable range. Increasing the amount of extract in either the creams or emulgels was observed to decrease the pH across both formulations although LGCM 2 showed significantly lower pH ($p < 0.05$). This can be attributed to the absence of triethanolamine (TEA) as TEA is widely used and known to balance and increase pH of cosmetic preparations. However, the pH range of all the formulations were found to be within an acceptable range. This result is similar to that of another study [38] where the

investigators reported near neutral pH (7.01 – 7.51) of gel formulations containing lemongrass oil.

Spreadability of topical preparations is a crucial parameter that is used to assess the ability to and the extent of spread after a particular time. This is important for patient compliance and uniform delivery of preparations because good spread ensures a greater skin surface area comes in contact with the preparations resulting in optimum absorption [39, 40]. High spreadability values signify that shorter time is required to achieve good spread and easy distribution of active substances [28]. Our results show that spreadability of the cream preparations (LGCM 1 and LGCM 2) were similar and those of the emulgels (LGEM 1 and LGEM 2) were also similar. However, the spreading ability of the cream formulations were significantly better (at $p < 0.05$) than those of the corresponding emulgel formulations. Nevertheless, both formulations showed good spreading ability which are comparable to reports of similar studies (18-76 – 25.65 g.cm/sec) involving emulgels prepared using lemongrass [41, 42]. Studies investigating other types of extracts have also reported high spreading ability of various topical formulations [43, 44].

Microbial load test. Microbial load of the repellent formulations is displayed in Table 4. The total aerobic microbial count was below 10^2 cfu/g specified for non-sterile formulations [45]. None of the preparations contained *Staphylococcus aureus* or *Pseudomonas aeruginosa* which are contraindicated in these types of preparation. Thus, the conditions set for microbial quality of these preparations were met and are free from contaminants. This indicates good hygienic process was ensued during the preparations, it also portrays good efficiency of the preservative incorporated in the formulations.

Skin irritation test. Skin sensitivity and/or irritation of the cream and emulgel

formulations assessed on laboratory rat skin is important in determining any potential risks associated with topical use of these formulations and also to give information as to the safety profile of these formulations [46]. Table 5 shows no erythema or oedema as the primary irritation index was zero (0), there was neither evidence of redness nor scratch marks on the animals. This indicates that the lemongrass formulations did not cause allergic reactions since erythema and oedema are one of the indicators of skin hypersensitivity or excessive immune response [47]. It is important that topical products do not cause irritation which could be discomforting leading to lack of compliance and to treatment failure. Our result show that the cream and emulgel formulations containing lemongrass extract is relatively safe and suitable for topical use. This is similar to an earlier study where citronella oil incorporated into cream formulations showed no irritation compared to the use of the oil alone [48].

Mosquito repellent assay. The repellent activity of the prepared formulations was determined using a modification of the proposed WHO model for use of rodents. Figure 1 shows the number of mosquito bites on the ventral skin of the laboratory rats over the period of study compared with bites on the untreated animals (negative control; NC).

Upon application of DEET, no bites were seen throughout the six hours, only two bites after five hours with LGEM 2 and 3 bites with LGCM 2. Larger number of bites; 10 and 9, were observed upon application of formulations LGEM 1 and LGCM 1 respectively. This shows that only LGEM 2 showed good repellence action against mosquito bites.

The percentage repellence activity ranged between 37.5 and 100% over the six hours period of study (Table 6). Each formulation had varying degree of repellent activity, which however, decreased with time across the formulations.

A minimum repellent impact of 80% has been reported to be ideal when investigating new repellent agents [49]. Formulation LGCM 1 showed good repellence action (81.3%) but only within three hours after application thereafter, the repellence action decreased progressively with time. The corresponding emulgel formulation (LGEM 1) on the other hand, showed good repellence action for about four hours after which the activity also decreased with time. Repellence action of LGCM 1 was significantly lower than that of LGEM 1 at $p < 0.05$. Formulations LGCM 2 and LGEM 2 showed good repellence action throughout the period of study since repellence action was $> 80\%$. This indicates higher concentration of extract exhibited better repellence action. Thus, formulations containing 1% of lemongrass extract (LGCM 1 and LGEM 1) were found to be less effective in repelling mosquitoes than those containing 2% of the extract (LGCM 2 and LGEM 2). This shows that increasing the concentration of lemongrass extract irrespective of the type of formulations is capable of providing better mosquito repellence action.

None of the formulations displayed repellence impact of 6 h as that of DEET. Only formulation LGEM 2 showed comparable action to that of the standard (DEET) for about 5 but at 6 h, the repellence action was significantly lower ($p < 0.05$) than that of DEET. Therefore, formulation LGEM 2 can be said to be the optimum formulation followed by LGCM 2, this suggests that LGCM 1 and LGEM 1 would require repeated application after three and four hours respectively to achieve a comparable sustained repellent activity as that observed with LGEM 2. Our results are similar to literature reports where good repellence action of cream and emulgel formulations containing different plant extracts have been reported [34, 44]. An earlier study investigated the use of crude plant extracts to prepare mosquito repellent cakes

and reported good repellence action of the herbal cakes [50].

Complete protection time is the time in which the first mosquito lands or bites the test organisms after application of the repellent product [51]. It is presumed that a product with long protection time and low percentage of biting has good repellence activity while that with short protection time even though the percentage biting is low is only good as a feeding deterrent [52]. Our results presented in Figure 2 show that LGEM 2 had the longest protection time (300 min) among the preparations been investigated; although this was significantly lower than that of the standard; DEET (480 min), followed BY LGCM 2 (240 min), LGEM 1 and LGCM 1 on the other hand, had the shortest protection time (30 and 20 min respectively). This shows that emulgel formulation containing 2% lemongrass extract is capable of offering complete mosquito repellence for up to five hours which shows good repellence activity and is comparable with reports of other studies where plant oils/extracts alone or in formulations have been investigated [53-55]. The activity of lemongrass as observed in this study may be attributed to the presence of its essential oil which has good insect repellent action.

The choice of proper dosage formulation is essential for patient's acceptability and therapeutic effectiveness. Formulations that are not aesthetically attractive and do not give the desired effectiveness loses its value in the array of dosage forms available to the patient. Emulgels have been described to offer good skin feel and a potential of enhanced activity; these were observed in this study. Formulation LGEM 2 had good physical properties and provided better repellence action over its corresponding cream formulation. Therefore, emulgels are suggested as preferred dosage form for delivery of Lemongrass extract for mosquito repellent activity.

Conclusion. This study has investigated the use of crude *n*-hexane extract of *Cymbopogon citratus* in formulation of cosmetically appealing, acceptable, safe and efficient mosquito-repelling products. Emulgel formulation containing 2 % concentration of *Cymbopogon citratus* extract, LGEM 2 is effective as a mosquito repellent offering about 5 h protection against mosquito bites. Thus, this natural and readily available plant remedy developed into a suitable dosage form can be employed as an alternative to or complement the currently available array of mosquito repellents.

REFERENCES

- World Health Organization (WHO). Malaria facts Sheet. <https://www.who.int/news-room/factsheets/detail/malaria>. 2022. Accessed on 4/10/2023.
- Das R, Pal P, Bhutia S. Pharmacognostical characterization and formulation of herbal-based low-cost mosquito repellents from *Elettaria cardamomum* (Linn.) seed by using natural binder. *Future Journal of Pharmaceutical Sciences*. 2021 Jan; 7(15):1-10. doi:10.1186/s43094-020-00166-3.
- Patel EK, Gupta A, Oswal RJ. A review on: mosquito repellent methods. *International Journal of Pharmaceutical, Chemical and Biological Sciences (IJPCBS)*. 2012; 2 (3):310-317.
- Dijendra RN, Ritobrata G, Ayantika P. The insect repellents: A silent environmental chemical toxicant to the health. *Environmental Toxicology and Pharmacology*. 2017 Mar; 50:91-102. doi:10.1016/j.etap.2017.01.019.
- Salunke MR, Bandal SC, Choudhari D, Gaikwad T, Dubey M. Review of herbal mosquito repellent. *International Journal of Scientific Development and Research*. 2022 Mar; 7(3):204-214.
- World Health Organization (WHO). Guidelines on safety monitoring of herbal medicines in pharmacovigilance systems; Geneva, Switzerland. World Health Organization. 2014. Accessed 19/02/2024.
- Olayemi O, John-Africa L, Abdullahi R, Isimi CY. Effect of emulgel containing *Newbouldia laevis* stem bark extract on croton-oil induced hemorrhoids. *Journal of Research in Pharmacy*. 2023; 27(4):1488-1498. doi:10.29228/jrp.435.
- Kaushik M, Yadav J, Singh A, Dubey MK. A systematic review of plant-based mosquito repellents and their activity. *Indian Journal of Natural Products and Resources*. 2023 Sept; 14(3):347-359. doi:10.56042/ijnpr.v14i3.4615.
- Moore SJ, Lenglet A, Hill N. Plant-based insect repellents. In *insect repellents: Principles methods, and use*. Boca Raton Florida: CRC Press; Debboun M, Frances SP, Strickman D. 2006: 1-15.
- Lawal OA, Avoseh NO, Ogunwande IA, Ogundajo AL. Chapter 18 - *Cymbopogon citratus* (V. Kuete, Ed.). *Medicinal spices and vegetables from Africa*. 2017: 397-423.
- Muanya C. Lemon grass induces childbirth-Features. *The Guardian Nigeria*. <https://guardian.ng/features/lemon-grass-induces-childbirth>. 2020. Accessed 10/11/2023.
- Adeniran OI, Fabiyi E. A cream formulation of an effective mosquito repellent: a topical product from lemongrass oil (*Cymbopogon citratus*) Stapf. *Journal of Natural Products and Plant Resources*. 2012; 2(2):322-327.
- Mukarram M, Choudhary S, Khan MA, Poltronieri P, Khan MMA, Ali J, Kurjak D, Shahid M. Lemongrass essential oil components with antimicrobial and anticancer activities. *Antioxidants (Basel)*. 2021 Dec; 11(20):1-23. doi:10.3390/antiox11010020.
- Sonibare MA, Okorie PN, Aremu TO, Adegoke A. Ethno-medicines for mosquito transmitted diseases from South-western Nigeria. *Journal of Natural Remedies*. 2015 Jan; 15(1):33-42. doi:10.18311/jnr/2015/470.
- Kujawska M, Pardo-de-Santayana M. Management of medicinally useful plants by European migrants in South America. *Journal of Ethnopharmacology*. 2015 Aug; 172:347-355. doi:10.1016/j.jep.2015.06.037.
- de Santana BF, Voeks RA, Funch LS. Ethnomedicinal survey of a maroon community in Brazil's Atlantic tropical forest. *Journal of Ethnopharmacology*. 2016 Apr; 181:37-49. doi:10.1016/j.jep.2016.01.014.
- Karami S, Yargholi A, Sadati LSN, Soleymani S, Shirbeigi L. A review of ethnopharmacology, phytochemistry and pharmacology of *Cymbopogon* species. *Research Journal of Pharmacognosy*. 2021 Jun; 8(3):83-112. doi:10.22127/RJP.2021.275223.1682.
- Okpo SO, Edeh I. A comprehensive review on lemongrass (*Cymbopogon citratus*) oil extraction and its applications. *EPRA International Journal of Research and Development (IJRD)*. 2022 Apr; 8(4):258-273. doi:10.36713/epra2016.
- Eden WT, Alighiri D, Supardi K, Cahyono E. The mosquito repellent activity of the active component of air freshener gel from java citronella oil (*cymbopogon winterianus*). *Journal of Parasitology*

- Research. 2020 Jan; 9053741. doi:10.1155/2020/9053741.
20. Oyedele AO, Gbolade AA, Sosan MB, Adewoyin FB, Soyelu OL, Orafidiya OO. Formulation of an effective mosquito-repellent topical product from Lemongrass oil. *Phytomedicine*. 2002 Apr; 9(3):259-262. doi:10.1078/0944-7113-00120.
 21. Ngan TTK, Hien TT, Danh PH, Nhan LTH, Tien LX. Formulation of the lemongrass (*Cymbopogon citratus*) essential oil-based eco-friendly diffuse solution. *IOP Conf. Series: Materials Science and Engineering*. 2020 Sept; 959:1-11. doi:10.1088/1757-899X/959/1/012024.
 22. Hazarika H, Krishnatreyya H, Tyagi V, Islam J, Gogoi N, Goyary D, Chattopadhyay P, Zaman K. The fabrication and assessment of mosquito repellent cream for outdoor protection. *Scientific Reports*. 2022 Feb; 12(2180):1-20. doi:10.1038/s41598-022-06185-9.
 23. Sundari PJ, Sushma C, Srija T. Development of herbal mosquito-repellent formulations and their comparative evaluation. *Asian Journal of Pharmaceutical and Clinical Research*. 2023 Jun; 16(6):127-132. Doi:10.22159/ajpcr.2023.v16i6.47274.
 24. Aditama W, Sitepu FP. The effect of lemongrass (*cymbopogon nardus*) extract as insecticide against *Aedes aegypti*. *International Journal of Mosquito Research*. 2019; 6(1):101-103.
 25. Rusnanda R, Ramadhini M, Putra WS, Asmaidi. Utilization of waste from refined lemongrass for producing mosquito repellent. *IOP Conf. Series: Earth and Environmental Science*. 2021; 644:1-4. doi:10.1088/1755-1315/644/1/012060.
 26. Chauhan L, Gupta S. Creams: A review on classification, preparation methods, evaluation and its applications. *Journal of Drug Delivery and Therapeutics*. 2002 Oct; 10(5-s):281-289. doi:10.22270/jddt.v10i5-s.4430.
 27. Suman D, Sangeeta and Beena K. Emulgel for topical drug delivery: A novel approach. *GSC Biological and Pharmaceutical Sciences*. 2020; 11(3):104-114. doi:10.30574/gscbps.2020.11.3.0165.
 28. Aremu O, Olayemi O, Ajala, T, Isimi Y, Oladosu P, Ekere K, John J, Emeje M. Antibacterial evaluation of acacia nilotica Lam (Mimosaceae) seed extract in dermatological preparations of the manuscript. *Journal of Pharmacy Research*. 2020 Jan; 24(1):170-181. doi:10.35333/jrp.2020.124.
 29. Pachauri A, Chitme H, Visht S, Chidrawar V, Mohammed N, Abdel-Wahab BA, Khateeb MM, Habeeb MS, Orabi MAA, Bakir MB. Permeability-enhanced liposomal emulgel formulation of 5-fluorouracil for the treatment of skin cancer. *Gels*. 2023 Mar; 9:209. doi:103390/gels9030209.
 30. OECD. Testing guide 404: OECD guidelines for testing of chemicals, Adopted guideline 404: Acute dermal irritation/corrosion. 2002.
 31. Banik B, Barman J, Dutta MP, Bhowmick N. Development and evaluation of herbal mosquito repellent cream. *Research Journal of Pharmacy and Technology*. 2021 Dec; 14(12):6262-6268. doi:10.52711/0974-360X.2021.01083.
 32. Maharaj R, Maharaj V, Newmarch M, Crouch NR, Bhagwandin N, Folb PI, Pillay P, Gayaram R. Evaluation of selected South African ethnomedicinal plants as mosquito repellents against the *Anopheles arabiensis* mosquito in a rodent mode. *Malaria Journal*. 2010; 9(301):1-8. doi:10.1186/1475-2875-9-301.
 33. World Health Organization (WHO). CTD/WHOPES/IC/96.1: Protocols for laboratory and field evaluation of Insecticides and Repellents. WHO/HQ Geneva. 1996.
 34. Sharma S, Neha I, Singh P, Singh A. Formulation and evaluation of mosquito repellent lotion of vasaka stem extract. *Annals of Romanian Society for Cell Biology*. 2021; 25(2):712-718.
 35. Husni P, Amalia AD, Mita SR, Putriana NA. Formulation and physical evaluation of cream containing neem oil 5 %. *Indonesian Journal of Pharmacy*. 2019 Oct; 1(3):79-83.
 36. Isa TS, Philippe B, Raymond H, Michel H, Jacques D. (2000). Improved kinetic parameter estimation in pH-profile data treatment. *International Journal of Pharmaceutics*. 2020 Apr; 198(1):39-49. doi:10.1016/S0378-517(99)00404-4.
 37. Sebamed. Skin pH - best pH level for skin and why is it important in skincare? Sebamed India. <https://www.sebamedindia.com/blog/how-important-is-ph-in-skincare-50>. Accessed on 17/10/2023.
 38. Gushit JS, Idoko O, Toekwal SJ, Idoko EJ, Enyia C. Formulation and evaluation of bio-mosquito repellent air freshener gel from oil extract of *Cymbopogon citratus* (DC.) Stapf (lemon grass) plant. *World Journal of Advanced Research and Reviews*. 2023 Jul; 19(1):496-507. doi.org/10.30574/wjarr.2023.19.1.0778.
 39. Nayeem N, Karvekar MD. Stability studies and evaluation of the semi-solid dosage form of the rutin, quercetin, ellagic acid, gallic acid and sitosterol isolated from the leaves of *Tectona grandis* for wound healing activity. *Archives of Applied Science Research*. 2011; 3(1):43-51.
 40. Deuschle VCK, Deuschle RAN, Bortoluzzi MR, Athayde ML. Physical chemistry evaluation of stability, spreadability, in vitro antioxidant, and photo-protective capacities of topical formulations containing *Calendula officinalis* L. leaf extract. *Brazilian Journal of Pharmaceutical Sciences*. 2015 Jan; 51(1):63-75. doi:10.1590/S1984-82502015000100007.

41. Kumar P, Bijauliya RK, Singh B, Yadav P, Khan WA. Formulation and evaluation of essential oil encapsulated mosquito repellent gel. *Journal of Drug Delivery and Therapeutics*. 2022 Jan; 12(1):23-29. doi:10.22270/jddt.v12i1.5265.
42. Sundari PJ, Sushma C, Srija T. Development of herbal mosquito-repellent formulations and their comparative evaluation. *Asian Journal of Pharmaceutical and Clinical Research*. 2023 Jun; 16(6):127-132. Doi:10.22159/ajpcr.2023.v16i6.47274.
43. Bhise M, Sudke S, Chaudhari P, Burakale P, Kandalkar A, Vasu S, Akarte A, Chandurkar P, Patil V, Bhajipale N, Pahuja S. Design and characterization of mosquito repellent emulgel formulations for circumventing infectious diseases. *Natural Volatiles and Essential Oils*. 2021; 8(6):3172-3189.
44. Jatkar P, Vrunal M, Mithun M, Waghmare V. Formulation and evaluation of herbal mosquito repellent cream. *European Chemical Bulletin*. 2023; 12(10):2105-2112.
45. United States Pharmacopeia (USP). General chapter on microbiological quality of non-sterile products: acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use. USP 41/NF39. 2019.
46. Adegboyega AE, Oluwalana IB. Structure, control and regulation of the formal market for medicinal Plant's products in Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines*. 2011; 8(3):370-376. doi:10.4314/ajtcam.v8i4.5.
47. James O, Sunday AB. Evaluation of acute dermal irritation and wound contraction by *Gymnema Sylvestr* and *Datura metel* extracts in rats. *American Journal of Biomedical and Life Sciences*. 2014 Aug; 2(4):83-88. doi:10.11648/j.ajbls.20140204.14.
48. Yadav NP, Rai VK, Mishra N, Sinha P, Bawankule DU, Pal A, Tripathi AK, Chanotiya CS. A novel approach for development and characterization of effective mosquito repellent cream formulation containing citronella oil. *BioMed Research International*. 2014 Oct; Article ID 786084: 1-11. doi:10.1155/2014/786084.
49. Maharaj R, Maharaj V, Newmarch M, Crouch NR, Bhagwandin N, Folb PI, Pillay P, Gayaram R. Evaluation of selected South African ethnomedicinal plants as mosquito repellents against the *Anopheles arabiensis* mosquito in a rodent mode. *Malaria Journal*. 2010 Oct; 9(301):1-8. doi.org/10.1186/1475-2875-9-301.
50. Baruah, P.S. and Borthakur, S.K. Formulation of an herbal mosquito repellent. *Annals of Plant Sciences*. 2016; 5(12):1463-1465. doi:10.21746/aps.2016.12.002.
51. World Health Organization (WHO). Guidelines for efficacy testing of mosquito repellents on human skin. iris.who.int/WHO_HTM_NTD_WHOPEPES_2009.4_eng.pdf. 2009. Accessed 20/02/2024.
52. Phasomkusolsil, S. and Soonwera, M. Insect repellent activity of medicinal plant oils against *aedes aegypti* (linn.), *anopheles minimus* (theobald) and *culex quinquefasciatus* based on protection time and biting rate. *South-East Asian Journal of Tropical Medicine and Public Health*. 2010 Jul; 41(4):831-840.
53. Wijayapala S, Vankar PS. Lantana oil and neem oil in combination with their extracts, a good combination for mosquito repellency. *International Journal of Collaborative Research on Internal Medicine and Public Health*. 2019; 11:917-923.
54. Kim SI, Tak JH, Seo JK, Park SR, Kim J, Boo KH. (2021). Repellency of Veratraldehyde (3,4-Dimethoxy benzaldehyde) against mosquito females and tick nymphs. *Applied Sciences*. 2021 May; 11(11):1-13. doi:10.3390/app11114861.
55. Gushit JS, Idoko O, Toekwal SJ, Idoko EJ, Enyia C. Formulation and evaluation of bio-mosquito repellent air freshener gel from oil extract of *Cymbopogon citratus* (DC.) Stapf (lemon grass) plant. *World Journal of Advanced Research and Reviews*. 2023; 19(1):496-507. doi:10.30574/wjarr.2023.19.1.0778.